Characterization of flocculation by *Halomonas* sp. B01

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Abstract. In order to improve flocculation activity of microbial flocculants in extreme environments, the resilience protective effect of compatible solute ectoine on the flocculation activity of flocculant was researched in this paper. Moderate halophilic bacterium *Halomonas* sp. B01 was isolated, screened and identified from the sediment of a saltern pool. The strain could synthesize flocculant and compatible solute ectoine simultaneously which were partially excreted into the fermentation broth during a fermentation process in this paper. The optimal conditions of flocculation activity by *Halomonas* sp. B01 flocculant were NaCl 30 g/L, 30 °C and pH 8. Ectoine had significant protective effects of resilience to extracted and purified flocculant in the high salt, high temperatures and alkaline environment. By applying Flocculant/Ectoine co-production fermentation broth by *Halomonas* sp. B01, the purification treatment of the desulfurized wastewater discharged from the seawater magnesia wet flue gas desulfurization was investigated, the results showed that the removal rate of solid content, turbidity and colority were 91.1%, 90.5% and 71.4%, respectively. The Flocculant/Ectoine co-production fermentation broth by *Halomonas* sp. B01 had significant effect of purification treatment to the desulfurized wastewater discharged from the seawater magnesia wet flue gas desulfurization.

Introduction

Compared to the traditional flocculants, microbial-produced bioflocculants are biodegradable, non-toxic and no secondary pollution. However, in industries such as petroleum, natural gas, seawater cooling and leather, the activity of microbial flocculants were reduced significantly because of high salinity, high or low temperatures, extreme pH and high concentrations of heavy metals etc.[1,2]. The researches on flocculation activity of microbial flocculants in extreme environments attract more attention in recent years.

Under environmental osmotic pressure stress, some moderate halophilic bacteria (such as *Halomonas*) could synthesize compatible solute ectoine (1, 4, 5, 6-tetrahydro-2-methyl-4-pyrimidine- carboxylic acid), which can mitigate deleterious effects of heat stress, freezing, drying, high salinity, oxygen radicals, radiation, urea and other denaturing agents on the integrity of proteins, nucleic acids, biomembranes and even whole cells [3]. It is reported that the ectoine-excreting *Halomonas* strain (*Halomonas* salina DSM 5928T) can synthesize and partially excrete ectoine, ectoine excreted to the extracellular can protect the extracellular macromolecules (such as polysaccharides, proteins, etc.) [4]. Based on this, an ectoine-excreting strain has flocculation activity was screened in this paper; the properties of strain flocculate were investigated in kaolin suspension system of 30 g/L NaCl; the resilience protective effect of ectoine on the flocculant was explored; on this basis, the flocculation activity of Flocculant/Ectoine co-production (simultaneous synthesis of flocculant and ectoine in a fermentation process) fermentation broth by the strain on the solid content, turbidity and colority of seawater magnesia wet flue gas desulfurization was investigated.

Materials and methods

Strain. The strain sample was isolated from the sediment of a saltern pool in Dalian, China.

Fermentation medium (g/L): glucose 40, $(NH_4)_2SO_4$ 10, yeast powder 1, KH_2PO_4 3, K_2HPO_4 9, $MgSO_4$•$7H_2O$ 0.4, $MnSO_4$•$4H_2O$ 0.01, NaCl 60. pH 7. The medium was sterilised at 121 °C for 20 min (Glucose was sterilized separately at 110 °C for 15 min).

Fermentation method. 1% strain activation suspensions were inoculated in the shake flasks (300 mL) containing 30 mL medium on a rotary shaker set at 120 rpm and 30 ℃ for 72 h.

Denitrification method of flocculation activity. The flocculation activity was determined by using spectrophotometric method [5] under 100 mL suspension contained 4%(w/v) kaolin with the 30 g/L NaCl. 721 spectrophotometer was used to calculate flocculant rate by absorbance at a wavelength of 550 nm. Flocculation activity was equal to flocculant rate. F=(A-B)×100%. F was flocculant rate; A was the contrast absorbance value; B was the contrast absorbance value contained flocculant.

Denitrification method of ectoine. The ectoine concentration was determined by using liquid chromatography method [4].

Denitrification method of turbidity. The turbidity was determined by using spectrophotometric method [6].

Denitrification method of colority. The colority was determined by Pt-Co colorimetric method [7].

Results and discussion

Isolation of Flocculant/Ectoine co-production strain. Flocculant/Ectoine co-production strain was isolated and screened from the sediment of a saltern pool in Dalian, China. The isolation was performed repeatedly by streak plate technique until the purified single colony was obtained. 20 single colonies were selected, each strain was cultured and fermented. The fermentation broth was centrifuged at 4 °C and 15000 × g for 15 min. The flocculation activity and concentrations of ectoine in fermentation broth were determined. Strain B01 had the highest flocculation activity and concentrations of ectoine which were 32.5% and 892.1 mg/L, respectively. The strain was identified by 16S rDNA analysis of *Halomonas* bacterium, and named as *Halomonas* sp. B01. *Halomonas* sp. B01 could synthesize flocculant and compatible solute ectoine simultaneously which were partially excreted into the fermentation broth during a fermentation process.

Focculation characterization of *Halomonas* sp. B01 flocculant. The flocculant was extracted and purified from *Halomonas* sp. B01 fermentation broth. A variety of extraction methods were investigated, and the flocculation activity of fermentation product extracted by the method according to the literature [8] was the highest, the extraction and preparation of flocculant were carried out by this method later. The flocculation activities of flocculant under different NaCl concentrations, temperature and pH were determined, respectively, the addition of flocculant was 0.6 g/L, as depicted in Fig. 1. The NaCl concentration in flocculation activity measurement system was adjusted to 0, 30, 60, 90, 120, 150, 180, 210 and 240 g/L. The flocculation activities were the highest (69.8% and 69.3%) when NaCl concentrations were 30 g/L and 60 g/L, the flocculation activity was 49% (70.2% of the highest flocculation activity) when NaCl concentration increased to 240 g/L (Fig. 1a). The strain had the highest flocculation activity under certain NaCl concentrations and the flocculation activity was less affected by high salt concentrations. The flocculant of strain B01 was salt tolerance flocculant. The temperature in flocculation activity measurement system was adjusted to 10, 20, 25, 30, 35, 40, 45, 50, 60 and 70 °C. The flocculation activities were higher (79.6%, 79.5% and 77.2%) at 30-40 °C, the flocculation activity was 13.2% when temperature increased to 70 °C (Fig. 1b). The optimum temperature was 30 °C. The pH in flocculation activity measurement system was adjusted to 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5 and 9 °C. The flocculation activities were higher (79.6%, 81.7%, 86% and 84.2%) when pH were 7-8.5, the flocculation activity was 60.5% when pH increased to 9 (Fig. 1c), pH was configured using Tris-HCl buffer in method above. The optimum pH was 8. The optimal conditions of flocculation activity by *Halomonas* sp. B01 flocculant were NaCl 30 g/L, 30 °C and pH 8.

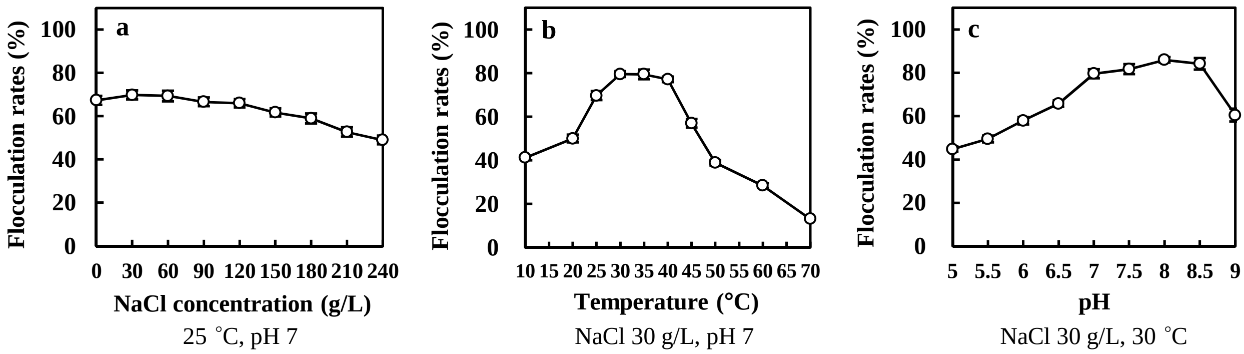

Fig. 1 Focculation characterization of *Halomonas* sp. B01 flocculant.

Resilience protective effect of ectoine on flocculant. In order to investigate the resilience protective effect of ectoine on flocculant, ectoine was added to flocculation activity measurement system. The concentrations of extracted and purified flocculant and ectoine were 0.6 g/L and 300 mg/L, respectively. The flocculation activities of flocculant and flocculant added ectoine were determined and the results were showed in Fig. 2. The flocculation activity increased by 43.3% (from 49% to 70.2%) under high salt condition (NaCl 240 g/L), the flocculation activity increased by 354.2% (from 13.2% to 59.8%) under high temperature condition (70 °C), the flocculation activity increased by 36% (from 60.5% to 82.2%) under alkaline condition (pH 9). The experimental results above showed that ectoine had significant protective effects of resilience to flocculant. The adverse conditions of high salinity, high temperature and alkaline lead to the changes in the spatial configuration of flocculant macromolecule, resulting in decreased flocculation activity [9]. The ectoine contained in the flocculant could enhance the stability of native conformation of flocculant under the inverse environment and then improved flocculation activity.

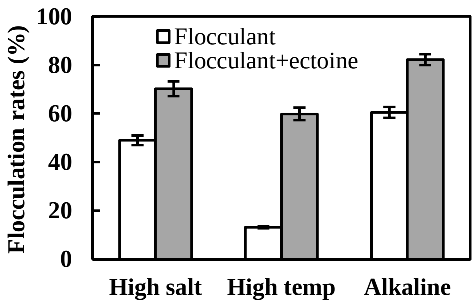

Fig. 2 Resilience protective effects of ectoine on flocculant.
Note: High salt (NaCl 240 g/L); High temperature (70 °C); Alkaline (pH 9).

The Flocculant/Ectoine co-production fermentation broth applied in extreme environment. Seawater magnesia wet flue gas desulfurization technology was applied to exhaust gas purification in cokes production, smelting, shipping and other industries. Desulfurized wastewater discharged from this treatment process has the characteristics of high salt, high temperature and high fine particle suspension. In this report, the desulfurized wastewater (Cl^- 47.3 g/L, 60 °C, pH 7.8, solid content 5.6 g/L) discharged from seawater magnesia wet flue gas desulfurization process were purified and treated. The Flocculant/Ectoine co-production fermentation broth was added to desulfurization wastewater (Addition 2%, flocculant concentration 0.3 g/L, ectoine concentration 17.8 mg/L). The solid content, turbidity and colority were determined and the results were showed in Table 1. As shown in Table 1, the removal rates of solid content, turbidity and colority were 91.1%, 90.5% and 71.4%, respectively. The Flocculant/Ectoine co-production fermentation broth by *Halomonas* sp. B01 had significant effect of purification treatment to the desulfurized wastewater discharged from the seawater magnesia wet flue gas desulfurization.

Table 1 Flocculation effect of Flocculant/Ectoine co-production fermentation broth on desulfurized wastewater

Flocculant	Solid content (g/l)	Turbidity (FTU)	Colority (mg/L Pt)
Before treatment	5.6±0.1	3701.1±23.9	1750
After treatment	0.5±0.1	350.2±9.9	500

Conclusions

Moderate halophilic bacterium *Halomonas* sp. B01 was isolated, screened and identified from the sediment of a saltern pool, could synthesize flocculant and compatible solute ectoine simultaneously which were partially excreted into the fermentation broth during a fermentation process in this paper. The optimal conditions of flocculation activity by *Halomonas* sp. B01 flocculant were NaCl 30 g/L, 30 °C and pH 8. Ectoine had significant protective effects of resilience to extracted and purified flocculant in the high salt, high temperatures and alkaline environment. By applying Flocculant/Ectoine co-production fermentation broth by *Halomonas* sp. B01, the purification treatment of the desulfurized wastewater discharged from the seawater magnesia wet flue gas desulfurization was investigated, the results showed that the removal rate of solid content, turbidity and colority were 91.1%, 90.5% and 71.4%, respectively. The Flocculant/Ectoine co-production fermentation broth by *Halomonas* sp. B01 had significant effect of purification treatment to the desulfurized wastewater discharged from the seawater magnesia wet flue gas desulfurization.

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